The Enzyme-catalysed Electrochemical Conversion of *p*-Cresol into *p*-Hydroxybenzaldehyde

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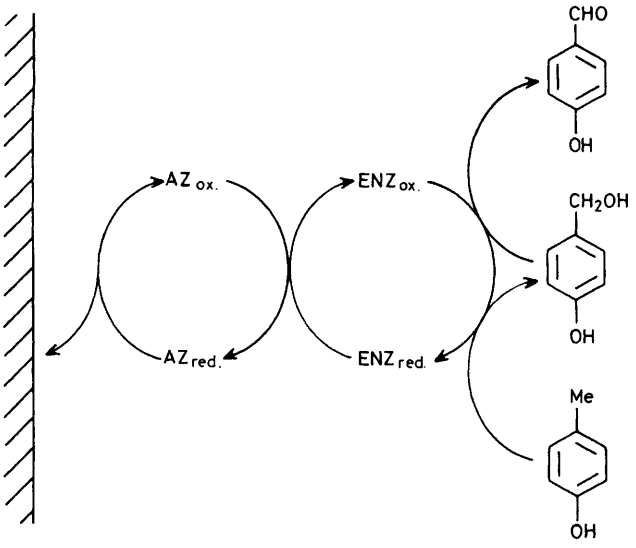
The electrochemical oxidation of *p*-cresol was effected enzymically, using either a blue copper protein, azurin, or ferroceneboronic acid as the mediator of the anodic reaction, giving *p*-hydroxybenzaldehyde as the only product.

There have been a number of recent developments in electrocatalysis and although significant improvements have been made, they generally lack selectivity and specificity.[1] These two qualities are important features which are commonly present in enzyme-catalysed reactions and thus it would seem of value to combine electrochemistry with enzyme catalysis.

We have described procedures which allow the observation of voltammetric responses of a number of redox proteins.[2,3] These redox proteins function, *in vivo*, as electron mediators to enzymes that catalyse oxidations or reductions. Thus, the ability to stimulate the direct electrochemical response of a redox protein can be exploited to couple an electrochemical device, through the redox protein, to the catalytic activity of an enzyme. This is exemplified by the coupling of cytochrome *c* to its natural partner, cytochrome oxidase, present in intact



Scheme 1

mitochondria.[4] An immediate application of electroenzymology has been the development of a variety of enzyme-based electroanalytical devices.[5] We now describe a bioelectrochemical system that may prove synthetically useful.

A number of hydroxylases have been isolated from bacteria of the *Pseudomonas* family which are effective catalysts for the oxidation of cresols and related phenols. In this conversion the methyl group is first oxidised to give the hydroxybenzyl alcohol, which can then act as the substrate for a further enzyme-catalysed oxidation to the aldehyde.[6] The blue copper protein azurin (Scheme 1: AZ) is thought to be the natural electron acceptor of this system.[7] We have found that the direct electrochemical response of azurin is well behaved at a number of electrodes, including pyrolytic graphite[3] and Au surface-modified with organic promoters, Figure 1a. The addition of either *p*-cresol methylhydroxylase (*e.g. from P. putida*) or *p*-cresol has no significant effect on the electrochemical response of azurin, Figure 1b. However, when *p*-cresol and the enzyme (Scheme 1: ENZ) are added to the azurin solution, an enhancement of the anodic response is seen (Figure 1c), which is more pronounced at low scan rates, diagnostic of a coupled-catalytic reaction,[8] Scheme 1.

An analysis of the dependence of the cyclic voltammetric response on scan rate and enzyme concentration, according to the procedure of Nicholson,[8] was used to derive an average second-order rate constant for the reaction between azurin and the enzyme of *ca.* 9×10^4 dm^3 mol^{-1} s^{-1} at 25 °C, pH 7.6.

It has been shown that ferrocenes can act as effective mediators to a variety of oxidoreductases.[5,9] As the half-wave potentials of some ferrocenes are not dissimilar from that of azurin ($E_{1/2}$ +307 mV *vs.* normal hydrogen electrode, N.H.E., pH 7.6) we considered it of value to replace azurin by a ferrocene as a mediator of electron transfer. The electrochemistry of ferroceneboronic acid ($E_{1/2}$ +323 mV *vs.* N.H.E., pH 7.6) is well behaved (Figure 2a) at a 'bare' gold electrode. The addition of the enzyme together with either *o*- or *m*-cresol does not effect the voltammetric response, Figure 2b. However, the addition of *p*-cresol to a solution containing the enzyme and ferroceneboronic acid leads to a large catalytic enhancement of the anodic response,† Figure 2c. The enzyme

† $K_3Fe(CN)_6$ and *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine were also evaluated as potential mediators. However, these couples gave no significance catalytic enhancement of the anodic response.

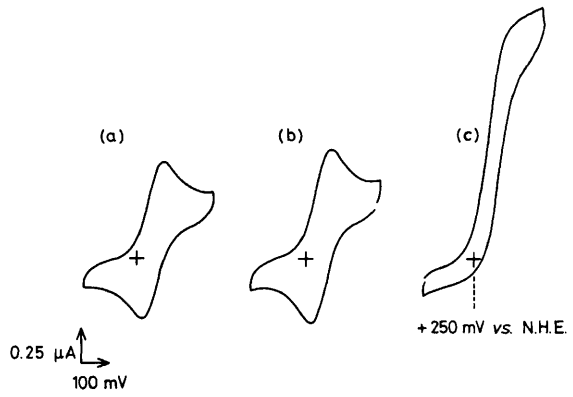


Figure 1. D.c. cyclic voltammograms (5 mV s^{-1}) recorded at a 4 mm diameter gold disc working electrode surface-modified with 2-(pyridinylmethylene)hydrazinecarbothioamide (2 min dip into a 0.5 mM solution followed by washing in distilled water). (a) 0.25 mM azurin in 50 mM Tris–HCl, 10 mM KCl, pH 7.6; (b), as (a), with the addition of *p*-cresol to a final concentration of 3 mM; (c), as (b), with the addition of *p*-cresol methylhydroxylase (from *P. putida*) to a final concentration of 0.7 μM.

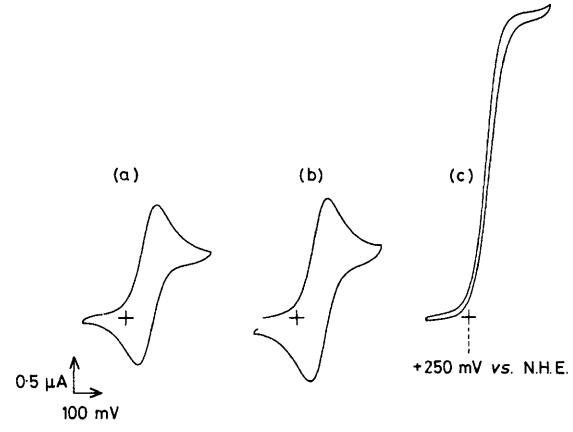


Figure 2. D.c. cyclic voltammograms (5 mV s^{-1}) recorded at a 4 mm diameter gold disc working electrode. (a) 0.25 mM ferroceneboronic acid in 50 mM Tris–HCl, 10 mM KCl, pH 7.6; (b), as (a), with the addition of *o*-cresol and *p*-cresol methylhydroxylase to final concentrations of 7 mM and 0.53 μM, respectively. (c), as (a), with the addition of *p*-cresol and *p*-cresol methylhydroxylase to final concentrations of 7 mM and 0.53 μM, respectively.

clearly demonstrates high selectivity among the various isomers of cresol. The average second-order rate constant derived from the reaction of the oxidised form of ferroceneboronic acid with the enzyme is *ca.* 5×10^5 dm^3 mol^{-1} s^{-1} at 25 °C, pH 7.6.

Given the ready availability of the ferrocene and its ease of use, we have exploited this mediator and developed a bulk electrosynthetic system‡ in which there is rapid (60 min at a

‡ Bulk electrosynthetic experiments were carried out in a two-compartment, stirred electrochemical cell (50 ml volume) containing large surface area gold (anode) and platinum (cathode) electrodes. The solutions used contained typically 1 mM ferroceneboronic acid in a basic support medium of 100 mM Tris[tris(hydroxymethyl)aminomethane]–HCl, 50 mM KCl, pH 7.6, containing 20% MeOH. The working electrode was poised at +600 mV *vs.* N.H.E.

current density of *ca.* 0.2 mA cm^{-2}) conversion of *p*-cresol (125 μmol) into *p*-hydroxybenzaldehyde by a catalytic quantity of the enzyme (90 nmol), under mild conditions (pH 7.6; 30 °C). H.p.l.c. analysis of a typical product mixture resulting from an electrosynthetic experiment confirms that there is complete turnover of the *p*-cresol. The yield of *p*-hydroxybenzaldehyde varies according to the source of the enzyme. Using *p*-cresol methylhydroxylase from *P. alcaligenes* yields were typically *ca.* 85% based on h.p.l.c. and coulometric analysis. It is possible that the current derived from the oxidation of *p*-cresol could be used to derive a second enzyme-controlled electrosynthetic reaction at the cathode.

We are grateful to the S.E.R.C. and Genetics International for financial support and Dr. M. J. Green for advice.

Received, 15th July 1985; Com. 1009

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